NOAA-NATIONAL MARINE FISHERIES SERVICE

SOUTHEAST FISHERIES SCIENCE LABORATORY IN CHARLESTON

FY95 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

Antibody probes directed against toxic algae are being evaluated using a highly sensitive, reporting system which involves the emission of light by antibodies bound to algal cells. In studies with laboratory cultures, this method can reliably detect as few as 10 algal cells in a sample. Tests with field samples are underway to confirm the antibody's ability to detect toxic algal species in a mixed phytoplankton assemblage. By using antibodies in conjunction with toxin receptor assays, it should be possible to obtain concurrent estimates of both algal cell and toxin concentrations in a water sample using simple boatside tests. It is anticipated that implementation of receptor assays will provide an effective means to monitor the toxicity of harmful algal blooms.

Studies on molecular mechanisms of growth regulation in toxic dinoflagellates have demonstrated that cell division is regulated by the eukaryotic cell cycle regulatory protein, CDC2 kinase. Molecular mechanisms by which the diurnal cycle entrains the cell division cycle of dinoflagellates are currently under laboratory investigation. A collaboration involving the Pittsburgh Zoo and the Smithsonian Museum has been initiated to investigate dynamics of *Gambierdiscus toxicus* bloom formation in a 5000 gallon coral reef microcosm. Cell cycle regulatory proteins in toxic dinoflagellates will yield useful probes for boatside tests to forecast the dynamics of harmful algal blooms.

New molecular forms of maitotoxin have been found in a Caribbean strain of the toxic algal, *Gambierdiscus toxicus,* using a rapid isoelectric focusing method. 1H NMR analysis determined that some of these maitotoxins have an eight member ether ring, yet exhibit differing side chains, molecular weights and possibly sugar moieties. API ionspray mass spectrometry has identified tentative masses ranging from 1200 to 5000 daltons. It has also demonstrated similar fragmentation spectra of the B portion of the Pacific form of maitotoxin for some of the purified isolates. Several ciguatoxins have also been isolated and final purification processes are being developed for these. API mass spectrometry has shown that MQ2 derived ciguatoxins may be larger than those previously reported. These studies provide groundwork for the production of toxin standards needed to manage the risk of ciguatera.

New, rapid, and inexpensive receptor-based assays for domoic acid and PSP toxins have been found to be reliable for detecting toxins in algae, shellfish, crab hepatopancreas, and the serum and urine of exposed humans and animals. These assays have been validated against HPLC analytical methods and the AOAC mouse bioassay. Collaborative field testing has been initiated with several state agencies responsible for seafood monitoring and inspection programs, in order to obtain side-by-side evaluation against the mouse bioassay. National reference laboratories within the European Community have requested that NMFS offer training workshops on implementing the receptor assays and expressed a desire to initiate collaborative testing programs. These assays are anticipated to be used in dockside testing of shellfish and confirmation of marine toxin exposure in seafood consumers.

The hazards of marine toxin exposure to seafood consumers have been further elucidated thorough the use of biomarkers. Two major brain regions have been identified to be the targets of amnesic shellfish poisoning; one controlling memory processing and the other regulating gastrointestinal function. Computer image analysis and reconstruction of this data base have generated three dimensional visualizations of brain damage caused by marine toxin exposure. Neonatal animals have been determined to be ten times more susceptible to amnesic shellfish poisoning. Amnesic shellfish poison (domoic acid) has been determined to be cleared from the serum within hours, and multiple exposures have been found not to change the clearance rate. Domoic acid given to lactating rats passes from the blood to the urine, but is found in very low concentrations in the milk. This indicates that although neonates are highly susceptible to the toxic effects of domoic acid, breast milk may not be a likely route of exposure. These studies are being used to better assess the risk of marine toxins to seafood consumers.